A New Pigment from *Tegula funebralis*

(Mollusca : Gastropoda)

BY

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(1 Text figure; 3 Tables)

In the trochacean species *Tegula funebralis* (A. Adams, 1854) and *T. brunnea* (Philippi, 1848) which are abundant in the intertidal zone of the Pacific coast, the female gonad is bright green. In both species, the pigment is found in droplets evenly dispersed throughout the yolk. An extraction of the pigment in *T. funebralis* was made. The crude green pigment was partitioned into a group of yellow carotenoids and an unknown green pigment. This is a study of these colored materials.

PREPARATION

The pigment was initially extracted from eggs carefully stripped from 200 gonads. The eggs were blended in a Waring blender with absolute methanol for five minutes. The methanol was changed, and extraction was repeated until the suspension was white. The crude green pigment was then dried and redissolved in methanol. This re-extraction was repeated three times. Ether and water partition of the pigment separated the material into a yellow epiphase and a green hypophase. Repeated partitioning was used to purify the materials.

RESULTS

The yellow material, dried and dissolved in petroleum ether (Bp. 40° 60° C) was placed on an Al_2O_3 column. The column was developed with a gradient of acetone in petroleum ether. The four bands observed were collected, and a tentative identification of zeaxanthin, lutein and alpha carotene was made from the data in Table I (P. Karrer & E. Jucker, 1950).

The green pigment could not be identified. Some of its properties are briefly stated in Table II. In addition, in aqueous solution it freely passed through sephadex G-75 which indicates a molecular size larger than that corresponding to a molecular weight of 40,000. In aqueous solution, it may be warmed to 100° C without obvious change. Prolonged heating at 100° C resulted in a brown discoloration, and is assumed to be due to decomposition. It is photosensitive, and becomes yellow with prolonged exposure to light. When reduced with hydrosulfite, a yellow color appears, but reoxidation to green can be achieved by autoxidation or treatment with H_2O_2. In methanol, a green fluorescence was observed in the oxidized form.

The large molecular size suggested a protein complex. The unknown was, therefore, placed in aqueous solution and an equal amount of $CHCl_3$ with .1 volume amylalcohol added. This was vigorously shaken and centrifuged for 10 minutes. A blue protein appeared between the phases of green aqueous solution and colorless $CHCl_3$. The aqueous phase was re-treated until the blue zone no longer appeared. The solubilities and spectrum of the un-

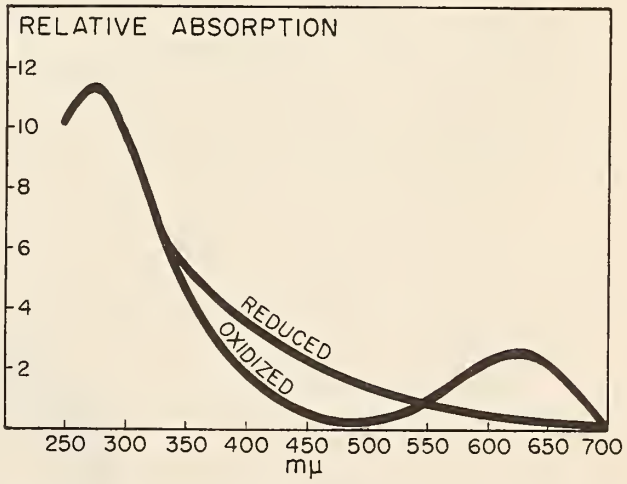

Figure 1: The absorption maxima of the unknown green pigment in H_2O. The oxidized state peaks at 640 and 273, while the reduced state peaks at 273μ.

Table 1

PROPERTIES OF YELLOW PIGMENTS

BAND	Absorption Maxima in CS_2	REMARKS	CAROTENOID*
I	517 482 450	hypophasic in petroleum ether and 90% MeOH	zeaxanthin
II	510 470 442	distributed in both phases of pet. ether and 90% MeOH	
III	508 475 445	hypophasic in petroleum ether and 90% MeOH	lutein
IV	509 477	epiphasic in petroleum ether and 90% MeOH	α - carotene

* Tentative identification on the basis of the absorption spectra and solubility properties.

Table 2

PROPERTIES OF GREEN PIGMENT

PIGMENT	SOLUBILITY	ABSORPTION MAXIMA mμ		COLORATION	
		oxidized	reduced	oxidized	reduced
1. Native	s. H_2O, MeOH, EtOH, acet. i. eth., $CHCl_3$ pet. eth., CS_2	H_2O 640 273 MeOH 640 370*(s)	H_2O ... 273	neutral-green acid-yellow alkaline-yellow with ppt.	neutral-yellow acid-yellow alkaline-yellow
2. Deproteinized	same as 1.	same as 1	same as 1	same as 1 except alkaline-yellow with no ppt.	same as 1
3. Allagochrome (Habermann, 1960)	s. H_2O	H_2O 630 320 260	H_2O 320 260	neutral-green acid-red alkaline-brown	neutral-yellow-orange acid- ? alkaline- ?

* (s) = shoulder.

s. = soluble
i. = insoluble
eth. = ether
pet. eth. = petroleum ether
acet. = acetone

Table 3

Results of mass spectral analysis of crude green pigment in percent by weight

ELEMENT	PERCENT	ELEMENT	PERCENT
Al	0.005	Na	1.0
Ca	0.10	Pb	0.01
Cu	0.005	Si	0.005
Fe	0.01	Sn	0.01
Mg	0.03	Zn	trace
Mn	trace		

known without protein appear in Table II. It should be noted that the absorption spectrum of the oxidized form was not changed.

In preliminary observations of the development of the closely related *Tegula brunnea* the green pigment present in the eggs was observed to concentrate in the ciliated cells of the trochophore. The amount appeared to increase as the velum formed.

DISCUSSION

The unknown pigment resembles Allagochrome (Habermann, 1960; Garrick & Habermann, 1962) in its absorption spectrum and oxidation-reduction activities. A comparison of the two pigments' properties can be seen in Table II. Allagochrome is present in a variety of higher plants and its function is hypothesized to be respiratory due to the ease with which oxidation and reduction can be induced.

The peak at 273 mμ also is suspiciously near the characteristic peak, 275 mμ of the coenzyme Q, a lipid soluble quinone (Crane, 1959). There is a broad distribution of the five known forms of coenzyme Q in aerobic tissues. It has been found in all vertebrates, higher plants, aerobic bacteria, invertebrates and red and green algæ. The view is that coenzyme Q is necessary for respiratory electron transfer occurring in the mitochondria and equivalent structures. While several quinones of biological origin have been described, their actual involvement in electron transport has seldom been demonstrated. If a respiratory function for the unknown pigment is postulated, the observed migration of the pigment to the ciliated cells of the velum of the trochophore and veliger larvæ may be of significance. These cells would be expected to have a high metabolic activity.

SUMMARY

The pigments of the eggs of *Tegula funebralis* were extracted in methanol. This crude green pigment contained three yellow materials with spectral properties resembling the carotenoids: zeaxanthin, lutein and alpha carotene, and an unknown green pigment. The green pigment was found to have an attached protein, absorption maxima in H_2O at 640, 273mμ in the oxidized form and 273 mμ in the reduced state, and a marked resemblance to known quinones that have been suggested to act as respiratory pigments.

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